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(54) Title: XYLANASES, NUCLEIC ADICS ENCODING THEM AND METHODS FOR MAKING AND USING THEM

(57) Abstract: The invention relates to xylanases and to polynucleotides encoding the xylanases. In addition, methods of designing new xylanases and methods of use thereof are also provided. The xylanases have increased activity and stability at increased pH and temperature.

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WO 2003/106654 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/19153

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/56, 9/24, 15/09, 15/11, 15/63, 1/21, 5/10
US CL : 536/23.2, 23.1, 24.3, 24.32, 24.33; 435/200, 320.1, 252.3, 325, 419, 6; 800/288, 295

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 536/23.2, 23.1, 24.3, 24.32, 24.33; 435/200, 320.1, 252.3, 325, 419, 6; 800/288, 295

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,916,795 A (FUKUNAGA et al.) 29 June 1999 (29.06.1999). see entire document.	40-47, 51-53, 99, 106, 107, 168-172, 197
A	CHEN, Y. Directed Evolution to Produce an Alkalophilic Variant From a Neocallimastix patriciarum Xylanase. Can. J. Microbiology Dec. 2001, Vol 47, pages 1088-1094.	1-37 40-47, 51-55, 99, 106, 107, 168-172, 197

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 May 2004 (14.05.2004)	22 JUN 2004
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Rebecca E. Prouty Telephone No. 571-272-1600

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US03/19153

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-37, 40-47, 51-55, 99, 106, 107, 168-172, 197 as drawn to SEQ ID NO:2

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups 1-190, claim(s) 1-37,40-47, 51-55, 99, 106, 107, 168-172, 197, 200, 209, 211, and 213, drawn to DNA, vectors and hosts cells encoding and the expression of the xylanase of SEQ ID NO:2 (Group 1), SEQ ID NO:4 (Group 2), SEQ ID NO:6 (Group 3), SEQ ID NO:8 (Group 4), SEQ ID NO:10 (Group 5), ... SEQ ID NO:380 (Group 190). Note claims 200, 209, and 211 include subject matter only of group 95 and claim 213 includes subject matter only of group 190.

Groups 191-380, claim(s) 38, 60-98, 175, 177, 180, 187, 188, 202, 204, 205, 210, 212, 214 and 215, drawn to the xylanase of SEQ ID NO:2 (Group 191), SEQ ID NO:4 (Group 192), SEQ ID NO:6 (Group 193), SEQ ID NO:8 (Group 194), SEQ ID NO:10 (Group 195), ... SEQ ID NO:380 (Group 380) and compositions thereof. Note claims 202, 210, and 212 include subject matter only of group 285, claim 214 includes subject matter only of group 380 and claim 215 includes subject matter only of groups 285 and 380.

Groups 381-570, claim(s) 48-49, drawn to transgenic animals encoding a xylanase of SEQ ID NO:2 (Group 381), SEQ ID NO:4 (Group 382), SEQ ID NO:6 (Group 383), SEQ ID NO:8 (Group 384), SEQ ID NO:10 (Group 385), ... SEQ ID NO:380 (Group 570).

Groups 571-760, claim(s) 56, drawn to methods of inhibiting translation of mRNA encoding the xylanase of SEQ ID NO:2 (Group 571), SEQ ID NO:4 (Group 572), SEQ ID NO:6 (Group 573), SEQ ID NO:8 (Group 574), SEQ ID NO:10 (Group 575), ... SEQ ID NO:380 (Group 760).

Groups 761-950, claim(s) 57-58, drawn to a double stranded inhibitory RNA comprising a portion of a nucleic acid encoding a xylanase of SEQ ID NO:2 (Group 761), SEQ ID NO:4 (Group 762), SEQ ID NO:6 (Group 763), SEQ ID NO:8 (Group 764), SEQ ID NO:10 (Group 765), ... SEQ ID NO:380 (Group 950).

Groups 951-1140, claim(s) 59, drawn to methods of inhibiting expression of a nucleic acid encoding a xylanase of SEQ ID NO:2 (Group 951), SEQ ID NO:4 (Group 952), SEQ ID NO:6 (Group 953), SEQ ID NO:8 (Group 954), SEQ ID NO:10 (Group 955), ... SEQ ID NO:380 (Group 1140).

Groups 1141-1330, claim(s) 100-102, 104, and 105, drawn to antibodies to a xylanase of SEQ ID NO:2 (Group 1141), SEQ ID NO:4 (Group 1142), SEQ ID NO:6 (Group 1143), SEQ ID NO:8 (Group 1144), SEQ ID NO:10 (Group 1145), ... SEQ ID NO:380 (Group 1330).

Groups 1331-1520, claim(s) 103, drawn to methods of identifying a xylanase of SEQ ID NO:2 (Group 1331), SEQ ID NO:4 (Group 1332), SEQ ID NO:6 (Group 1333), SEQ ID NO:8 (Group 1334), SEQ ID NO:10 (Group 1335), ... SEQ ID NO:380 (Group 1520) by immunodetection.

Groups 1521-1710, claim(s) 108, drawn to methods of identifying a xylanase of SEQ ID NO:2 (Group 1521), SEQ ID NO:4 (Group 1522), SEQ ID NO:6 (Group 1523), SEQ ID NO:8 (Group 1524), SEQ ID NO:10 (Group 1525), ... SEQ ID NO:380 (Group 1710) by change in substrate or product concentrations.

Groups 1711-1900, claim(s) 109, drawn to methods of identifying substrates of the xylanase of SEQ ID NO:2 (Group 1711), SEQ ID NO:4 (Group 1712), SEQ ID NO:6 (Group 1713), SEQ ID NO:8 (Group 1714), SEQ ID NO:10 (Group 1715), ... SEQ ID NO:380 (Group 1900).

Groups 1901-2090, claim(s) 110-111, drawn to methods of identifying compounds that bind to the xylanase of SEQ ID NO:2 (Group 1901), SEQ ID NO:4 (Group 1902), SEQ ID NO:6 (Group 1903), SEQ ID NO:8 (Group 1904), SEQ ID NO:10 (Group 1905), ... SEQ ID NO:380 (Group 2090).

Groups 2091-2280, claim(s) 112-115, drawn to methods of identifying modulators of the activity of the xylanase of SEQ ID NO:2 (Group 2091), SEQ ID NO:4 (Group 2092), SEQ ID NO:6 (Group 2093), SEQ ID NO:8 (Group 2094), SEQ ID NO:10 (Group 2095), ... SEQ ID NO:380 (Group 2280).

Groups 2281-2470, claim(s) 116-120, drawn to a computer and computer readable medium having a sequence of a xylanase of SEQ ID NO:2 (Group 2281), SEQ ID NO:4 (Group 2282), SEQ ID NO:6 (Group 2283), SEQ ID NO:8 (Group 2284), SEQ ID NO:10 (Group 2285), ... SEQ ID NO:380 (Group 2470) or a nucleic acid encoding said xylanase stored thereon.

Groups 2471-2660, claim(s) 121-125, drawn to methods of computer analysis of polynucleotide sequences encoding the xylanase of SEQ ID NO:2 (Group 2471), SEQ ID NO:4 (Group 2472), SEQ ID NO:6 (Group 2473), SEQ ID NO:8 (Group 2474), SEQ ID NO:10 (Group 2475), ... SEQ ID NO:380 (Group 2660).

Groups 2661-2850, claim(s) 39 and 126-127, drawn to methods of isolating a nucleic acid encoding the xylanase of SEQ ID NO:2 (Group 2661), SEQ ID NO:4 (Group 2662), SEQ ID NO:6 (Group 2663), SEQ ID NO:8 (Group 2664), SEQ ID NO:10 (Group 2665), ... SEQ ID NO:380 (Group 2850) by amplification.

Groups 2851-3040, claim(s) 128-130, drawn to methods of isolating a nucleic acid encoding by the xylanase of SEQ ID NO:2 (Group 2851), SEQ ID NO:4 (Group 2852), SEQ ID NO:6 (Group 2853), SEQ ID NO:8 (Group 2854), SEQ ID NO:10 (Group 2855), ... SEQ ID NO:380 (Group 3040) by hybridization.

Groups 3041-3230, claim(s) 131-140 and 201, drawn to methods of generating a variant polynucleotide of a polynucleotide encoding the xylanase of SEQ ID NO:2 (Group 3041), SEQ ID NO:4 (Group 3042), SEQ ID NO:6 (Group 3043), SEQ ID NO:8 (Group 3044), SEQ ID NO:10 (Group 3045), ... SEQ ID NO:380 (Group 3230). Note claim 201 includes subject matter only of group 3135.

Groups 3231-3420, claim(s) 141-145, drawn to methods of modifying codons of a nucleic acid encoding the xylanase of SEQ ID NO:2 (Group 3231), SEQ ID NO:4 (Group 3232), SEQ ID NO:6 (Group 3233), SEQ ID NO:8 (Group 3234), SEQ ID NO:10 (Group 3235), ... SEQ ID NO:380 (Group 3420).

Groups 3421-3610, claim(s) 146-149, drawn to methods of making a library of nucleic acids encoding variants of the xylanase of SEQ ID NO:2 (Group 3421), SEQ ID NO:4 (Group 3422), SEQ ID NO:6 (Group 3423), SEQ ID NO:8 (Group 3424), SEQ ID NO:10 (Group 3425), ... SEQ ID NO:380 (Group 3610).

Groups 3611-3800, claim(s) 150-155, drawn to methods of making a small molecule using the xylanase of SEQ ID NO:2 (Group 3611), SEQ ID NO:4 (Group 3612), SEQ ID NO:6 (Group 3613), SEQ ID NO:8 (Group 3614), SEQ ID NO:10 (Group 3615), ... SEQ ID NO:380 (Group 3880).

Groups 3881-3990, claim(s) 156-157, drawn to methods of determining a functional fragment of the xylanase of SEQ ID NO:2 (Group 3881), SEQ ID NO:4 (Group 3882), SEQ ID NO:6 (Group 3883), SEQ ID NO:8 (Group 3884), SEQ ID NO:10 (Group 3885), ... SEQ ID NO:380 (Group 3990).

Groups 3991-4180, claim(s) 158-161, drawn to methods of whole cell engineering by real-time metabolic flux analysis using a cell encoding the xylanase of SEQ ID NO:2 (Group 3991), SEQ ID NO:4 (Group 3992), SEQ ID NO:6 (Group 3993), SEQ ID NO:8 (Group 3994), SEQ ID NO:10 (Group 3995), ... SEQ ID NO:380 (Group 4180).

Groups 4181-4370, claim(s) 162-166, a signal sequence from the xylanase of SEQ ID NO:2 (Group 4181), SEQ ID NO:4 (Group 4182), SEQ ID NO:6 (Group 4183), SEQ ID NO:8 (Group 4184), SEQ ID NO:10 (Group 4185), ... SEQ ID NO:380 (Group 4370) or fusion proteins thereof.

Groups 4371-4560, claim(s) 167, drawn to methods of increasing the thermostability of the xylanase of SEQ ID NO:2 (Group 4371), SEQ ID NO:4 (Group 4372), SEQ ID NO:6 (Group 4373), SEQ ID NO:8 (Group 4374), SEQ ID NO:10 (Group 4375), ... SEQ ID NO:380 (Group 4560).

Groups 4561-4750, claim(s) 173-174, drawn to methods of using the xylanase of SEQ ID NO:2 (Group 4561), SEQ ID NO:4 (Group 4562), SEQ ID NO:6 (Group 4563), SEQ ID NO:8 (Group 4564), SEQ ID NO:10 (Group 4565), ... SEQ ID NO:380 (Group 4750) to hydrolyze xylan.

Groups 4751-4940, claim(s) 176, drawn to methods of dough conditioning using the xylanase of SEQ ID NO:2 (Group 4751), SEQ ID NO:4 (Group 4752), SEQ ID NO:6 (Group 4753), SEQ ID NO:8 (Group 4754), SEQ ID NO:10 (Group 4755), ... SEQ ID NO:380 (Group 4940).

Groups 4941-5130, claim(s) 178-179, drawn to methods of beverage production using the xylanase of SEQ ID NO:2 (Group 4941), SEQ ID NO:4 (Group 4942), SEQ ID NO:6 (Group 4943), SEQ ID NO:8 (Group 4944), SEQ ID NO:10 (Group 4945), ... SEQ ID NO:380 (Group 5130).

Groups 5131-5320, claim(s) 181-186, drawn to methods of administration of a nutritional supplement comprising the xylanase of SEQ ID NO:2 (Group 5131), SEQ ID NO:4 (Group 5132), SEQ ID NO:6 (Group 5133), SEQ ID NO:8 (Group 5134), SEQ ID NO:10 (Group 5135), ... SEQ ID NO:380 (Group 5320).

Groups 5321-5510, claim(s) 189-196, drawn to methods of delivering a xylanase supplement comprising the xylanase of SEQ ID NO:2 (Group 5321), SEQ ID NO:4 (Group 5322), SEQ ID NO:6 (Group 5323), SEQ ID NO:8 (Group 5324), SEQ ID NO:10 (Group 5325), ... SEQ ID NO:380 (Group 5510).

Groups 5511-5700, claim(s) 203, drawn to methods of reducing the lignin content of wood using the xylanase of SEQ ID NO:2 (Group 5511), SEQ ID NO:4 (Group 5512), SEQ ID NO:6 (Group 55123), SEQ ID NO:8 (Group 5514), SEQ ID NO:10 (Group 5515), ... SEQ ID NO:380 (Group 5700).

Groups 5701-5890, claim(s) 206-208, drawn to methods of eliminating a microorganism using the xylanase of SEQ ID NO:2 (Group 5701), SEQ ID NO:4 (Group 5702), SEQ ID NO:6 (Group 5703), SEQ ID NO:8 (Group 5704), SEQ ID NO:10 (Group 5705), ... SEQ ID NO:380 (Group 5890).

The inventions listed as Groups 1-5890 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The only shared technical feature among the DNAs of Groups 1-190 is that they all encode xylanases. Similarly, the only shared technical feature among the proteins of Groups 191-380 is that they have xylanase activity. However, these shared technical features are not special technical features as defined by PCT Rule 13.2 as xylanases and nucleic acids encoding xylanases were taught in the prior art (see for example US Patent 5,916,795). The DNAs of Group 1-190, the proteins of Groups 191-380, the transgenic animals of Groups 381-570, the double stranded inhibitory RNAs of Groups 761-950, the antibodies of Groups 1141-1330, the computers of Groups 2281-2470 and the signal sequences of Groups 4181-4370 do not share a corresponding special technical feature even though the DNA encodes the protein because the prior art clearly teaches genes which encode xylanases including signal sequences thereof are known in the art (see above) and each of these products comprise chemically unrelated structures. Therefore, the only shared technical feature linking these of these claims does not constitute a special technical feature as defined in PCT Rule 13.2 as it is not a feature which defines a contribution the claimed invention makes over the prior art. The methods of Groups 571-760, 951-1140, 1331-2280, 2471-4180, and 4371-5890 do not share any technical feature as they comprise unrelated steps and produce unrelated effects. The methods of Groups 571-760, 1901-2090, and 2471-4180 do not share any technical feature with Groups 191-570, 761-950, 1141-1330, 2281-2470 and 4181-4370 and do not have unity of invention with Groups 1-190 as Groups 1-190 already includes a method of use of the DNA which comprises unrelated steps to the methods of Groups 571-760, 1901-2090, and 2471-4180 and PCT Rule 13.2 does not provide for the inclusion of multiple methods of use within the main invention. The methods of Groups 951-1140, 1331-1900, 2091-2280 and 4371-5890 do not share any technical feature with Groups 1-190.

Continuation of B. FIELDS SEARCHED Item 3:
EAST, MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, CAS, NTIS, ESBIOBASE, BIOTECHNO, WPI, search terms: xylanase#, alkali?, thermostab?